

**Appl. No.** : **10/023,275**  
**Filed** : **Dec 13 2001**

**AMENDMENTS TO THE SPECIFICATION**

**Please replace Tables 1 and 2 at pages 16-19 with the following amended Tables 1 and 2 (to replace □ which is not reproduced below with  $\mu$ ):**

TABLE 1

Apparent kinetic parameters for putrescine incorporation into SPR2, involucrin, and succinylated casein by

0.94 pmol membrane-bound and solubilized TGase 1\*

	$V_{max}$	$k_{cat}(\text{putrescine})$	$K_M(\text{app})$	$K_{cat}/K_M$
	$\mu\text{mol}\cdot\text{min}^{-1}$	$\text{min}^{-1}$	$\mu\text{M}$	$\text{min}^{-1}\cdot\mu\text{M}^{-1}$
<u>Solubilized TGase 1 with</u>				
1. Putrescine (+ 1 $\mu\text{M}$			221 $\pm$ 34	
succinylated casein)				
Succinylated casein	12.7 $\pm$ 2.3	13.5	61.4 $\pm$ 9.2	220 $\pm$ 44
Human SPR2	10.4 $\pm$ 1.7	11.1	13.2 $\pm$ 1.9	838 $\pm$ 107
Involucrin	482 $\pm$ 37	513	114 $\pm$ 19	4514 $\pm$ 1073
<u>SLV-bound TGase 1 (0% PS) with</u>				
Putrescine (+ 1 $\mu\text{M}$			397 $\pm$ 49	
Succinylated casein)				

Succinylated casein	3 ± 0.3	3.2	138 ± 18.6	23.2 ± 6
Human SPR2	11.2 ± 1.4	11.9	25.3 ± 2.6	470 ± 61
<b><u>SLV-bound TGase 1 (15% PS) with</u></b>				
Succinylated casein	3.2 ± 0.2	3.4	145 ± 18	23 ± 5
Human SPR2	13.1 ± 1.9	13.9	22.8 ± 3	610 ± 87
Involucrin	2.4 ± 0.4	2.55	2.9 ± 0.4	879 ± 92

\*The PS content of the SLV is shown in parentheses.  $KM_{app}$  values pertain to the protein,  $V_{max}$  and  $K_{cat}$  data are that of putrescine incorporation.

TABLE 2

*Glutamine Residues in Involucrin That Serve as Acyl Donors by TGase 1\**

	Peak	Peptide sequence position in involucrin	Modified Q residue(s) (mol/mol)
<i>Panel A: TGase 1 reaction in the absence of membranes:</i>			
5			
10	4	375-378	Q375 (0.40)
	5	532-537	Q535 (0.08)
	6	116-121	Q117 (0.66), Q118 (0.23)
	7	122-127	Q122 (0.25)
15	8	379-384	Q379 (0.04), Q382 (0.05)
	11	295-3-1	Q298 (0.08)
	15	101-109	Q106 (0.06), Q107 (0.46)
	16	165-174 and 522-531	<u>Q168</u> or Q525 (0.09), <u>Q172</u> or Q529 (0.37)
	17	432-441	Q439 (0.45)
20	18	385-394	<u>Q392</u> (0.32)
	19	395-404	Q398 (0.08), Q402 (0.39)
	20	285-294	<u>Q288</u> (0.07), <u>Q292</u> (0.35)
	21	36-46	Q45 (0.16)
	22	129-140	Q132 (0.06), Q133 (0.47)
25	23	69-80	Q73 (0.04)
	25	509-521	Q515 (0.06), Q519 (0.35)
	26	419-431	Q419 (0.36), Q425 (0.08), Q429 (0.39)
	27	469-481	Q469 (0.17), Q475 (0.06), Q479 (0.42)
	28	563-575	Q572 (0.10)
30	29	405-418	Q408 (0.07), Q412 (0.41), Q415 (0.08)
	31	150-164	Q151 (0.30), <u>Q158</u> (0.09)
	32	486-501	<u>Q489</u> (0.49), <u>Q495</u> (0.11), <u>Q496</u> (0.71), Q499 (0.04)
35	33	442-461	Q445 (0.09), Q449 (0.43), <u>Q455</u> (0.10), <u>Q456</u> (0.56), Q459 (0.04)
	34	324-344	<u>Q328</u> (0.10), Q332 (0.38), Q335 (0.25), <u>Q342</u> (0.37)
	35	265-284	Q272 (0.34), Q282 (0.45)
	36	81-100	Q88 (0.07)
40	38	538-562	Q539 (0.42), Q551 (0.48)
	39	345-374	Q348 (0.09), Q353 (0.33), Q363 (0.35), <u>Q368</u> (0.09), <u>Q369</u> (0.79), Q372 (0.37)
	40	175-264	<u>Q178</u> (0.09), Q182 (0.42), <u>Q188</u> (0.08), Q192 (0.36), Q198 (0.09), Q202 (0.37), <u>Q208</u> (0.09), Q212 (0.35), Q218 (0.08), Q222 (0.36), Q227 (0.10), Q232 (0.29),
45			

Q238 (0.06), Q242 (0.34), Q248 (0.08),  
Q252 (0.35), Q258 (0.07), Q262 (0.37)

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*Panel B: TGase 1 reaction with Sf9 particulate fraction:*

10	6	116-121	Q118 (0.03)
	7	122-127	Q122 (0.07)
	15	101-109	Q107 (0.09)
	22	129-140	Q133 (0.04)
	32	486-501	<u>Q496</u> (0.54)

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*Panel C: TGase 1 reaction on SLV:*

20	6	116-121	Q118 (0.04)
	7	122-127	Q122 (0.06)
	15	101-109	Q107 (0.11)
	22	129-140	Q133 (0.16)
	32	486-501	<u>Q496</u> (0.59)

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\*Tryptic peptide peaks that contained <sup>14</sup>C-putrescine label, indicated by arrows in FIGURE 9, were sequenced. The location and amount of modified Gln (Q) residues were identified by the appearance and quantitation of PTH-(glutamyl)putrescine (FIGURE 8). Underlined residues denote those seen in *in vivo* cross-linking. (Steinert, P.M. and L.N. Marekov, *J. Biol. Chem.*, 272: 2021-2030 (1997)).

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**Please replace the paragraph at 46:13-29 with the following amended paragraph (to excise embedded hyperlinks which are not reproduced below):**

By using this computational protocol, genome sequence data bases such as maintained by various organizations including: [tigr.org/tdb](http://tigr.org/tdb), [genetics.wisc.edu](http://genetics.wisc.edu), [stanford.edu/~ball](http://stanford.edu/~ball), [hiv-web.lanl.gov](http://hiv-web.lanl.gov), [ncbi.nlm.nih.gov](http://ncbi.nlm.nih.gov), [ebi.ac.uk](http://ebi.ac.uk), [patteur.fr/other/biology](http://patteur.fr/other/biology), and [genome.wi.mit.edu](http://genome.wi.mit.edu), can be rapidly screened for specific protein active sites and for identification of the residues at those active sites that resemble a desired molecule. Several other groups have developed databases of short sequence patterns or motifs designed to identify a given function or activity of a protein. These databases, notably [Prosite \(expasy.hcuge.ch/sprot/prosite\)](http://expasy.hcuge.ch/sprot/prosite), [Blocks \(blocks.fhcrc.org\)](http://blocks.fhcrc.org), and [Prints \(biochem.ucl.ac.uk/bsm/dbfrowser/PRINTS/PRINTS\)](http://biochem.ucl.ac.uk/bsm/dbfrowser/PRINTS/PRINTS), use short stretches of sequence information to identify sequence patterns that are specific for a given function; thus they avoid the problems arising from the necessity of matching entire sequences. In this manner, new enzymes and adaptors are rationally selected for further identification by carrier system characterization assays, as described above. Rounds or cycles of functional assays on the molecules and derivatives thereof and further FFF refinement and database searching can be done.

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**Please replace the paragraph at 49:17-29 with the following amended paragraph (to replace ☐ which is not reproduced below with prime):**

The nucleic acid encoding the polypeptide to be expressed can be obtained by PCR from the bacterial vector using oligonucleotide primers complementary to the nucleic acid and containing restriction endonuclease sequences for Pst I incorporated into the 5 prime primer and BglII at the 5 prime end of the corresponding cDNA 3 prime primer, taking care to ensure that the nucleic acid is positioned in frame with the poly A signal. The purified fragment obtained from the resulting PCR reaction is digested with PstI, blunt ended with an exonuclease, digested with Bgl II, purified and ligated to pXT1, now containing a poly A signal and digested with BglII. The ligated product is transfected into a suitable cell line, e.g., mouse NIH 3T3 cells, using Lipofectin (Life Technologies, Inc., Grand Island, New York) under conditions outlined in the product specification. Positive transfectants are selected after growing the transfected cells in 600ug/ml G418 (Sigma, St. Louis, Missouri). Preferably the expressed protein is released into the culture medium, thereby facilitating purification.